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TITLE: Method for determining concentration of substances such as glucose in blood - useful in biosensors

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BASIC-ABSTRACT:

NOVELTY - The concentration level of the desired substance such as glucose, cholesterol, is measured by computing the ratio of the measured electric current in the sample to its time differential.

USE - In biosensor for measuring levels of glucose, cholesterol, etc in blood sample.

ADVANTAGE - Discriminates between control liquid sample and actual blood sample and displays the result, thereby preventing incorrect recognition by the user.

CHOSEN-DRAWING: Dwg.1/2

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(54)【発明の名称】　サンプル弁別の方法

(57)【要約】

【課題】　バイオセンサシステムによる分析対象物質の測定では、バイオセンサシステムの応答が正常であるか否かの確認をコントロール液を用いて行っているが、その際に使用者がコントロール液と血液を弁別することなく、バイオセンサシステムが自動的に両者の区別を行うことのできる方法を提供する。

【解決手段】　バイオセンサシステムによって測定される電流とその時間微分の比を算出することにより、予めシステムに記憶された所定の比較値と比較することでコントロール液と血液を弁別することが可能となった。

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【特許請求の範囲】

【請求項1】　電流を測定することによって液体サンプルの分析対象物濃度を定量するバイオセンサシステムに用いられる方法であって、測定した電流とその時間微分の比を算出することを特徴とするサンプル弁別の方法。

【請求項2】　前記測定した電流とその時間微分の比を複数回算出することを特徴とする請求項1に記載のサンプル弁別の方法。

【請求項3】　前記複数回算出された比の任意の2つの差を算出することを特徴とる請求項2に記載のサンプル弁別の方法。

【請求項4】　前記算出された比を予めシステムに記憶された所定の比較値と比較し、その結果を表示することを特徴とする請求項1に記載のサンプル弁別の方法。

【請求項5】　前記複数回算出された比をそれぞれ予めシステムに記憶された所定の比較値と比較し、その結果を表示することを特徴とする請求項2に記載のサンプル弁別の方法。

【請求項6】　前記算出された複数の比の任意の2つの差を予めシステムに記憶された所定の比較値と比較し、その結果を表示することを特徴とする請求項3に記載のサンプル弁別の方法。

【請求項7】　前記液体サンプルが血液あるいはコントロール液である請求項1から6のいずれかに記載のサンプル弁別の方法。

【請求項8】　コントロール液によるシステムチェックの判定結果を表示することを特徴とする請求項4から7のいずれかに記載のサンプル弁別の方法。

【発明の詳細な説明】

【0001】

【発明の属する技術分野】本発明は、液体サンプルに含まれる分析対象物濃度を測定するための方法に関する。特に血液などの体液に含まれる例えばグルコース、コレステロールなどの濃度を電流測定により定量するための方法に関する。

【0002】

【従来の技術】従来、液体サンプル、特に血液などの生体サンプル中の分析対象物濃度を簡便、迅速に定量するものとしてバイオセンサが知られている。

【0003】バイオセンサとは、電気化学測定を行うためのデバイスの1種である。電気化学測定とは、その名の通り、化学的反応を電気的な信号、例えば電流、電圧、電荷量として測定するものである。電気化学測定の弱点は、その特異性の低さにある。すなわち、化学的反応によって生成した物質が電気化学測定可能な物質であっても、測定サンプル中に電気化学測定可能な別の物質が存在すると、直ちに誤差を生じてしまうのである。バイオセンサは、この電気化学的測定の特異性の低さという弱点を補うために、分析対象物と選択的かつ特異的に反応し得る生体機能物質、例えば酵素などを利用している。バイオセンサは、少なくとも次の2つの部分を有する。1つは選択機能部として、目的物と選択的かつ特異的に反応する部分である。例えば酵素や抗体がこれに相当する。もう1つは信号変換部として、選択機能部の化学的反応を電気的な信号へと変換する部分である。主として電極に相当する。バイオセンサを用いた分析対象物の測定は、直接的に電流、電圧、電荷量などの各種電気的な量が測定対象になり得る。この中でも電流を測定対象とするバイオセンサが多く存在している。

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【0004】バイオセンサシステムとは、バイオセンサへ電位を与えたり、バイオセンサの信号である電流を測定したりする機器と、テストセルとしてのバイオセンサを組み合わせて使用するものである。例えば、血液サンプル中の乳酸濃度を定量するためのバイオセンサシステムであれば、乳酸と特異的に反応する乳酸オキシダーゼ（LOD）、乳酸デヒドロゲナーゼ（LDH）などの酵素を選択機能部とするバイオセンサをテストセルとして、血液サンプルと前記酵素などとの反応が始まってからの時間を計時するタイマー機能、定められた時間経過後に定められた電位をバイオセンサに印加する機能、前記電位印加開始から定められた時間経過後に電流を測定する機能、電流と乳酸濃度との相関関係、例えば検量線を記憶する機能、前記記憶された相関関係、例えば検量線と前記測定された電流から乳酸濃度を判定する機能、前記判定した結果をディスプレイなどに表示する機能等を有する機器と組み合わせて使用することで構築することが出来る。このようなバイオセンサシステムは、血液サンプル中の乳酸濃度の定量にとどまらず、種々のものが知られている。

【0005】欧州特許出願公開第0230472号には、電流測定を利用して血液サンプルのグルコース濃度を測定するバイオセンサシステムが開示されている。このシステムでは、測定電極、リファレンス電極およびカウンタ電極を備えたバイオセンサがテストセルとして用いられている。前記電極は、グルコースオキシダーゼ、フェリシアン化カリウムおよびその他の成分を含有する試薬層で覆われている。血液サンプルを試薬層に接触させて入れると、サンプル中のグルコースが、グルコースオキシダーゼの作用を介してフェリシアン化カリウムと反応し、フェロシアン化カリウムを形成する。その後で電極に電圧を印加すると、逆反応が生じて、最初の反応で生じたフェロシアン化カリウムの濃度に比例した電流が流れる。この電流の測定値がサンプル中のグルコースの濃度に対応するとしている。

【0006】電気化学測定において、時間に対して矩形波的に電位を印加し、それに対する電流を測定する手法は、ポテンシャルステップ法として一般的に知られている。ここで言う「時間に対して矩形波的な電位印加」とは、実質的に瞬間にある一定電位を印加し、その後前記一定電位を印加し続けるという意味である。このような

ポテンシャルステップ法では、電位を印加してからの時間の経過と共に減衰していく電流、減衰電流が観察される。この減衰電流は、時間に依存しているので、時間の関数として表すことが出来る。他方この減衰電流は、分析対象物濃度にも依存している。ゆえに、この減衰電流は分析対象物濃度の関数としても表すことが出来る。減衰電流は、時間と分析対象物濃度の関数として表せるわけである。従って、減衰電流に影響を与えるその他の因子として、例えばサンプルの性状、サンプル温度、電極面積、印加電位などが一定に保たれている場合には、減衰電流をＩ、時間をｔ、分析対象物濃度をＣとした時、Ｉ＝（ｔ，Ｃ）と表すことが出来る。Ｉ（ｔ，Ｃ）はＩがｔ，Ｃの関数であることを表しており、時間ｔを一定に保つことが出来れば、言い換えれば、同一のタイミングで測定された減衰電流は、分析対象物濃度のみの関数として表すことが出来る。式で表すと、Ｉ（ｔ=一定，Ｃ）＝Ｉ（Ｃ）となる。従来のバイオセンサシステムでは、この原理が用いられている。予め電流測定の時間を決めておき、その時の電流と分析対象物濃度の相関関係を示す検量線Ｉ（Ｃ）を使って、電流と分析対象物質濃度を関連づけているのである。この時、電流測定は１回のみである。

【０００７】複数回の電流測定を行うバイオセンサシステムが、特許公報第２６５１２７８号に開示されている。この発明では、電流測定を複数回行うことで得られた情報を異常なサンプル供給状態の検知の為に活用している。分析対象物濃度以外の情報を、電流の大きさそのものではなく、異なる時刻における電流間の関係から得ようとしている。言い替えれば、電流Ｉよりは寧ろ、時間の関数Ｉ（ｔ）から情報を得ようとする試みである。このシステムでは、連続する２回の電流測定を行い、両者の測定電流の比とその測定が行われた時間の平方根の逆数の比とを比較することによって、システムが正常に働いているかどうかの指標を得ている。これは、サンプル量半無限、電極に電位を加える直前の状態で分析対象物濃度分布一様、サンプル撹拌無し、という条件下でのポテンシャルステップ法におけるＩ（ｔ、Ｃ）の理論式が時間（ｔ）の平方根の逆数に比例していることに基づく。例えば、血液サンプルが検出電極の表面全体を覆わない場合、テスト前またはテスト中に反応域が水和した場合、さらに、リークが生じて血液サンプルが反応域内の電極だけでなく反応域の外部をも覆う場合には読取り値にエラーが生じる場合には前記連続する２回の測定で得られた電流の比とその測定が行われた時間の平方根の逆数の比とを比較することで得られた指標により、エラーが生じていることを判別することができるとしている。

【０００８】バイオセンサシステムが正常に働いているかどうかをチェックするための他の従来技術としては、コントロール液を用いる方法が知られている。この技術

は、特許公報第２６５１２７８号開示の発明とはその目的が多少異なり、主にバイオセンサシステムにおける機器が正常に働いているかどうかのチェックを目的としている。以下に、血液サンプルのグルコース濃度を定量するためのバイオセンサシステムを例として、コントロール液を用いたシステムチェックの方法を説明する。

【０００９】コントロール液は既知のグルコース濃度に調製されており、正常なシステムがどのような応答、例えばグルコース濃度表示をするのかが予め調べられている。更にコントロール液には、血液サンプルの性状に近づけるために、水溶性高分子を加えて粘性を増しているものや、色素が加えられているものもある。システム使用者は、定期的に、あるいは必要に応じて、前記コントロール液を血液サンプルを測定するときと同様の手段で測定する。そして、システムの応答が正常なものであるかを判定する。システムの応答が正常であるか否かの判定は、コントロール液の容器や添付文書などに表示された正常範囲と実際のシステム応答値を比較することに依る。例えば、正常範囲が７０～１２０ｍｇ／ｄＬであるコントロール液を測定したとき、システムの応答値が８０ｍｇ／ｄＬ、１１０ｍｇ／ｄＬなどであればシステムは正常に働いていると判定し、システムの応答値が５０ｍｇ／ｄＬ、１４０ｍｇ／ｄＬ等であれば、システムは異常であると判定する。このようにして、バイオセンサシステムが正常に働いているかどうかを判定するわけである。

【００１０】

【発明が解決しようとする課題】上記のようにコントロール液を用いたバイオセンサシステムのチェック方法では、システムチェックのためにコントロール液を測定する場合であっても、血液サンプルなどの通常サンプルの測定であっても、システムの応答としては分析対象物質濃度の表示があるだけで同一であるため、使用者がサンプルとして何を用いたか認識していなければ、コントロール液を測定した結果であるのか、血液サンプルなどの通常サンプルを測定した結果であるのかを判別できないという問題があった。このため、例えば、血液サンプルなどの通常サンプルを測定した結果であるにも関わらずコントロール液を測定した結果であると誤認識して、又はシステムが正常であるにもかかわらず異常であると判定してしまったり、又はシステムが異常であるにもかかわらず正常であると判定してしまったり、或いはコントロール液を測定した結果であるにも関わらず血液サンプルなどの通常サンプルを測定した結果であると誤認識して、医師が間違った診断を下したりしてしまう恐れがあった。特に後者のような場合では、誤った血液検査データを基に誤った診断が下される危険性を孕み、重大であった。

【００１１】また、システムが正常であるかどうかを判定する際にも、コントロール液容器や添付文書等に表示

された正常範囲とコントロール液の測定結果を比較する必要があり、作業が煩雑であった。頻回にわたってシステムチェックを行わなければならない場合、又は複数濃度のコントロール液を用いてシステムチェックを行う場合、或いは多数のバイオセンサシステムに対してシステムチェック行わなければならない場合などで特に煩雑であった。

【００１２】本発明の目的は、使用者に負担を掛けず、コントロール液を測定した際にはシステムが自動的に、血液サンプルなどの通常サンプルではなく、コントロール液であることを弁別してこれを表示し、使用者側の誤認識を防ぐことの出来るバイオセンサシステムを構築するための方法を提供する。併せて、煩雑な作業を伴うこと無しにコントロール液によるシステムチェックの結果を知ることの出来るバイオセンサシステムを構築するための方法を提供することである。

【００１３】

【課題を解決するための手段】本発明は、電流を測定することによって液体サンプルの分析対象物濃度を定量するバイオセンサシステムに用いられる方法であって、測定した電流とその時間微分の比を算出し、これを指標としてサンプル弁別を行う方法である。前記測定した電流の時間微分は、アナログ的に連続する測定データから得られる厳密な意味での微分であって良いし、デジタル的に不連続である測定データから実質的に微分値となるように算出されたものであっても良い。例えば、比較的短い時間の間隔で電流を測定し、両者の差を持って時間微分とすることが出来る。つまり、時間微分の定義式、ｌｉｍ（Δｔ→０）＝｛Ｉ（ｔ＋Δｔ）－Ｉ（ｔ）｝／Δｔでは、Δｔは無限小でなければならないので、不連続な測定から厳密な時間微分を求めることは不可能であるが、比較的短い時間の間隔Δｔで測定した電流から近似的に時間微分を求めることは出来るということである。また、何らかの理由でΔｔをそれほど短く出来ない場合には、例えば、３点以上の測定を行い、それらの差の移動平均を取るなどして、近似的に時間微分を求めることが出来る。こうして得られた時間微分と電流の比を取って、サンプル弁別の指標とする訳である。時間微分と電流の比は、所望により、微分／電流であっても良いし、電流／微分であっても良く、これらに四則演算的に定数を含めても良い。それらには本質的な違いはなく、後に述べる比較値の選び方が異なるだけである。時間微分は、厳密な微分（ｄＩ／ｄｔ）であっても、近似的な微分（ΔＩ／Δｔ）であっても良く、サンプル弁別の指標を算出する際にも、電流と時間微分の比が、｛Ｉ／（ｄＩ／ｄｔ）｝又は｛Ｉ／（ΔＩ／Δｔ）｝であっても、｛（ｄＩ／ｄｔ）／Ｉ｝又は｛（ΔＩ／Δｔ）／Ｉ｝でも良いし、これらの比をα、ａ、ｂをある定数として、（ａ　α±ｂ）などとして良い。何故なら、上記のどれをサンプル弁別の指標として選んでも本質的な違いはなく、後に述べる比較値の取り方が異なってくるだけだからである。

【００１４】サンプル弁別のための他の指標を得るための方法として、前記電流とその時間微分の比を複数回算出して、それらの差を取ることもできる。電流とその時間微分の比αをｎ個（ｎ回）算出したとして、そのｋ番目、１番目をα$_k$，α$_1$とし、これらの差をΔαとすれば、Δα（ｋ，１）＝α$_k$－α$_1$と表すこともできる。但し、Δα（ｋ，１）の括弧は、ｋ番目と１番目のαの差であることを表す。この場合も、サンプル弁別の指標を得るのに符号を変更しても差し支えなく、Δα（ｋ，１）＝α$_k$－α$_1$であっても、Δα（ｋ，１）＝α$_1$－α$_k$であっても良い。また、四則演算的に定数を含めても良く、ｃ、ｄをある定数として、｛ｃ（Δα）±ｄ｝をサンプル弁別のための指標としても良い。何故なら、上記のどれをサンプル弁別の指標として選んでも本質的な違いはなく、後に述べる比較値の取り方が異なってくるだけだからである。

【００１５】これまで説明してきたような方法で得られた指標を比較値と比較することにより、サンプルを弁別することが出来る。前記比較値は、実験的に求められる値であるので、本法を用いてサンプルを弁別するには、事前にサンプルがどのような指標値を取るのか調べておかなければならない。例えば、指標として電流とその時間微分の比αを用いるとしてサンプルＡとサンプルＢを弁別する際には、サンプルＡでα$_0$未満，サンプルＢでα$_0$以上のα値が観察されるというような閾値としてα$_0$を比較値に選んでおけば良い。このようにして決められた比較値をシステムに予め記憶させておけば、実際の測定時に観察される指標値と比較値との比較によってサンプル弁別が可能となる。弁別が可能であれば、その結果を前記システムに表示させることは容易である。

【００１６】測定される液体サンプルが血液またはコントロール液による場合には、本法を用いてコントロール液であることを弁別した上で、システムチェックの判定結果をも併せて表示することが出来る。判定結果の表示は、○や×、ＯＫやＮＧなど二者択一式にしても良いし、コントロールＨｉｇｈ・２７０ｍｇ／ｄｌ・ＯＫなど具体的に表示しても良い。

【００１７】

【発明の実施の形態】

【実施例】以下、具体的な実施例により本発明を更に詳しく説明する。バイオセンサシステムの一例として、血液サンプルのグルコース濃度を定量するグルコースセンサシステムについて説明する。グルコースセンサシステムのテストセルであるグルコースセンサは、次のような構成のものを使用した。ＰＥＴ（ポリエチレンテレフタレート）からなる絶縁性の基板上に、スクリーン印刷によって、リード（銀）と作用極、対極を含む電極系（カーボン）及び電気絶縁層が形成されている。電気絶縁層

は、作用極及び対極の露出部分の面積を一定とし、かつリードを部分的に覆っている。このようにして形成された電極部分上に、親水性高分子であるカルボキシメチルセルロース（CMC）層が形成されている。更にこのCMC層の上に、酵素としてのグルコースオキシダーゼ（GOD）と電子伝達体（メディエータ）としてフェリシアン化カリウムからなる、酵素及びメディエータ層が形成されている。（以下CMC層と酵素及びメディエータ層を併せて反応層と称する。）更に、カバーとスペーサーからなるインサートが形成されており、インサートへサンプルが触れると、毛管現象によって、反応層および電極系へ一定量として約3μLのサンプルが供給されるようになっている。一方、測定機器としては、汎用のポテンショスタット、BAS100B／W（BAS製）を使用した。通常のバイオセンサシステムであれば、一つのテストセルにそれ専用の機器が組み合わされて使用されるが、本実施例では汎用の機器を使用している。しかしながら、既知技術を用いた簡易な機器であっても、本実施例の再現実施は容易である。

【0018】測定サンプルは、コントロール液1、コントロール液2、血液を用いた。コントロール液1は、防腐剤として安息香酸、色素として赤色二号とグルコースが添加された水溶液である。グルコース濃度は、97mg／dlと325mg／dlの2種類を用いた。コントロール液1の性状は、水に近く、比較的粘性が低かった。コントロール液2は、水溶性高分子であるポリビニルピロリドン（PVP）とグルコースの水溶液である。グルコース濃度は、83mg／dlと256mg／dlの2種類を用いた。コントロール液2の性状は、比較的粘性が高かった。血液は、血球成分濃度とグルコース濃度の異なる6種類を用いた。血球成分濃度（ヘマトクリット値で示す。）とグルコース濃度は、（30%，102mg／dl）、（44%，101mg／dl）、（58%，103mg／dl）、（28%，268mg／dl）、（45%，263mg／dl）、（62%，266mg／dl）であった。また、抗凝固剤としてヘパリ＊ンナトリウムを添加した。これらのサンプルをテストセルの反応層および電極系へ供給して、25秒間電位を印加せずに静置した。この間、サンプル中のグルコースが、グルコースオキシダーゼの作用を介して反応層のフェリシアン化カリウムと反応し、フェロシアン化カリウムを生成する。そして、25秒後から5秒間、作用極と対極の間に500mVの一定電位を、時間に対して矩形波的に印加し、0．1秒間隔で電流を測定した。この時観察される電流は、フェロシアン化カリウムからフェリシアン化カリウムという逆反応によるものである。この電流は、最初の反応で生じたフェロシアン化カリウムの濃度に比例しているので、この電流の測定値がサンプルのグルコース濃度に対応する。また、この電流は、電位印加からの時間経過に伴って減衰する減衰電流である。図1に、時間と印加電位の関係を示す。また、図2に、コントロール液1のグルコース濃度97mg／dlであるものを測定した際の減衰電流を図示する。横軸の時間単位は秒で、電位が印加された瞬間を0秒としている。

【0019】（演算1）以下に示す方法で、サンプル弁別のための演算値を得る。尚、本実施例では電圧印加からの時間が4秒後の電流を用いているが、これに限定されることはなく、サンプル弁別に適した時間の電流を利用することが出来る。先ず、測定した減衰電流の4秒後の値から4．1秒後の電流を減じた差を取り、4秒後電流の時間微分とした。これは、時間微分の定義式、$\lim_{\Delta t \to 0} \{I(t+\Delta t) - I(t)\} / \Delta t$ における、無限小のΔtに代えて、$\Delta t = 0.1$秒を取り、符号を入れ替えたものに相当する。こうして得られた時間微分を分母、4秒後電流を分子とした比の値を取り、これを弁別指標α_4にした。α_4の添え字の4は、4秒後電流から得られた指標であることを示す。表1に各サンプルにおける指標α_4の値を示す。尚、コントロール液1およびコントロール液2のデータは、それぞれ、測定数30の結果であり、血液のデータは、測定数90の結果である。表1にサンプル別のα_4値を示す。

【表1】

	平均値	最小値	最大値
コントロール液1	122	114	134
コントロール液2	75	65	85
血液	83	54	104

表1からは、コントロール液1の最小値の方が血液の最大値よりも大きいため、例えば、比較値として110を選べば、比較値110よりも大きいα_4値を示すものをコントロール液1、小さいものを血液として両者を弁別することが出来る。同様にして、コントロール液1とコントロール液2も弁別可能である。しかし、コントロール液2と血液では、コントロール液2の最大値が血液の最小値よりも大きいために、弁別が不能である。

【0020】（演算2）次は、演算1と同様にして得られる電流とその時間微分の比α_t（t秒後電流から得ら※れるαの意）を複数求め、それらの差をサンプル弁別のための指標値とする場合について説明する。0．5秒、2秒について、$\alpha_{0.5}$，α_2を求める。式で表せば、それぞれ、$\alpha_{0.5} = I_{0.5} / (I_{0.5} - I_{0.6})$

$\alpha_2 = I_2 / (I_2 - I_{2.1})$と表せる。これらを求めておいて、次に、これらの差を取る。本実施例では、α_2から$\alpha_{0.5}$を減じた差を取った。t_2秒のα_{t2}とt_1秒のα_{t1}の差を$\Delta\alpha(t_1, t_2)$のように表すとして、本実施例での演算を式で表すと、$\Delta\alpha(0.5, 2) = \alpha_2 - \alpha_{0.5} = \{I_2 / (I_2 - I_{2.1})\} - \{I_{0.5} / (I_{0.5} -$

$I_{0.6}$）｝となる。表2に各サンプルにおけるΔα（0．5，2）の値を示す。尚、基としたデータは、演算1に用いたものと同一で、コントロール液2のデータ＊は、測定数30の結果であり、血液のデータは、測定数90の結果である。

【表2】

	平均値	最小値	最大値
コントロール液1	42	39	44
コントロール液2	25	21	29
血液	35	33	39

表2からは、コントロール液2の最大値の方が血液の最小値よりも小さいため、例えば、比較値として30を選べば、比較値30よりも小さいΔα（0．5，2）値を示すものをコントロール液2、大きいものを血液として両者を弁別することが出来る。

【0021】以上の結果から、コントロール液1，コントロール液2および血液の3サンプルを弁別することが可能となる。すなわち、本実施例に於いては、Δα（0．5，2）の値が30未満であればコントロール液2であると判定し、30以上であれば血液あるいはコントロール1であると判定し、更にα_4の値が110未満であれば血液で110以上であればコントロール液1であると判定することが出来る。これにより、コントロール液1，コントロール液2および血液の3サンプルを弁別出来たことになる。

【0022】これまでに述べてきた様な方法によって、サンプル弁別が可能となるので、弁別結果を表示することの出来るバイオセンサシステムを構築するのは容易である。また、コントロール液を用いたシステムチェックの結果表示は、本法によって測定されたサンプルがコン※トロール液であることが弁別できるので、予めシステムにコントロール液の正常範囲電流値を記憶させておけば、濃度を調べるための電流測定の結果とその正常範囲電流値を比較した結果を表示することも可能になる。

【0023】

【発明の効果】本発明の方法を用いることによって、使用者に負担を掛けずコントロール液を測定した際には機器が自動的に血液サンプルなどの通常サンプルではなく、コントロール液であることを弁別してこれを表示し、使用者側の誤認識を防ぐことの出来るバイオセンサシステムを構築することが出来る。併せて、煩雑な作業を伴うこと無しにコントロール液によるシステムチェックの結果を知ることの出来るバイオセンサシステムを構築することが出来る。

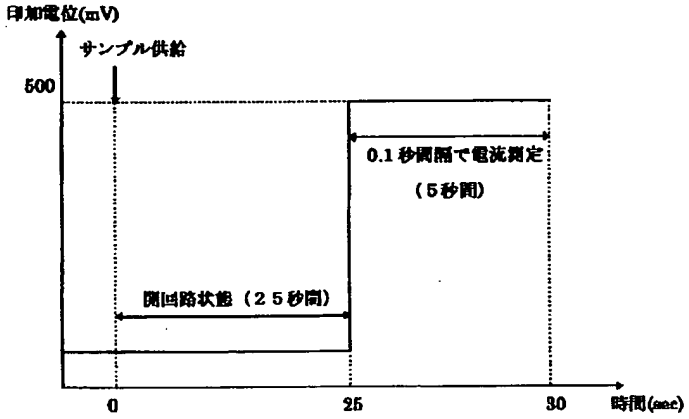
【0024】

【図面の簡単な説明】

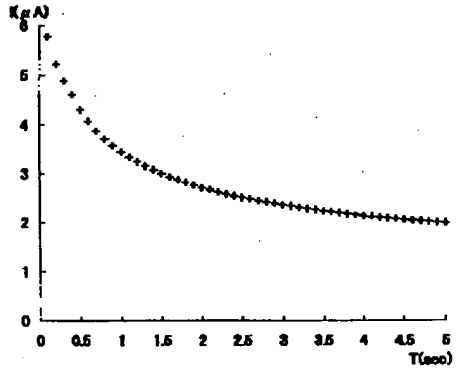
【図1】　時間と印加電位の関係を表すものである。

【図2】　グルコース濃度97mg／dlであるものを測定した際の減衰電流を示す。

【図1】



【図2】



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INOUE YOICHI

(54) SAMPLE DISCRIMINATING METHOD

(57)Abstract:
PROBLEM TO BE SOLVED: To establish a method for automatically discriminating a control liquid at the time of measuring a control liquid and preventing error recognition on the side of a user, by computing a ratio between a measured current and its time differentiation and discriminating a sample with the computed ratio as an index.
SOLUTION: The control liquids 1 and 2 to be measured and blood of a sample are supplied for the reaction layer and an electrode system of a test cell and are placed still for 25 seconds without impressing a potential. For 5 seconds after the 25 seconds, a controlled potential of 50 V is impressed between a working electrode and a counter electrode to measure a current at intervals of 0.1 second. The measured value of the current corresponds to the glucose concentration of the sample. In addition, the current is a transient-decay current associated with the passage of time from the impression of a potential. The difference obtained by subtracting a measured transient-decay current, for example, after 4 seconds from the value of a measured transient decay current after 4 seconds is taken as the time differentiation of the current after 4 seconds, and the value of a ratio with the time differentiation as a denominator and the current after 4 seconds as a numerator is taken as a discrimination index. It is possible to discriminate three samples of the control liquids 1 and 2 and blood from the discrimination index.

LEGAL STATUS

[Date of request for examination]

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* NOTICES *

1. This document has been translated by computer.So the translation may not reflect the original precisely.
2. **** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
[Claim 1] The method of the sample discrimination characterized by computing the ratio of the current which is the method used for the biosensor system which carries out a fixed quantity, and measured the analysis object concentration of a liquid sample by measuring current, and its time differential.
[Claim 2] The method of the sample discrimination according to claim 1 characterized by carrying out multiple-times calculation of the ratio of the current which carried out [aforementioned] measurement, and its time differential.
[Claim 3] It is the method of sample discrimination given in the feature and ******** 2 about computing two differences with the arbitrary ratio by which multiple-times calculation was carried out [aforementioned].
[Claim 4] The method of the sample discrimination according to claim 1 characterized by displaying the result as compared with the predetermined comparison value beforehand memorized by the system in the ratio by which calculation was carried out [aforementioned].
[Claim 5] The method of the sample discrimination according to claim 2 characterized by displaying the result as compared with the predetermined comparison value beforehand memorized by the system in the ratio by which multiple-times calculation was carried out [aforementioned], respectively.
[Claim 6] The method of the sample discrimination according to claim 3 characterized by displaying the result as compared with the predetermined comparison value beforehand memorized by the system in two differences with two or more arbitrary ratios by which calculation was carried out [aforementioned].
[Claim 7] The method of sample discrimination given in either of the claims 1-6 whose aforementioned liquid samples are blood or control liquid.
[Claim 8] The method of sample discrimination given in either of the claims 4-7 characterized by displaying the judgment result of a system check with control liquid.

[Translation done.]

* NOTICES *

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[The technical field to which invention belongs] this invention relates to the method for measuring the analysis object concentration contained in a liquid sample. It is related with the method for [which carries out the fixed quantity of the concentration, such as a glucose and cholesterol for example by the amperometry] being contained especially in body fluid, such as blood.
[0002]
[Description of the Prior Art] Conventionally, the biosensor is known as what carries out the fixed quantity of the analysis object concentration in living body samples, such as a liquid sample, especially blood, simple and quickly.
[0003] A biosensor is one sort of the device for performing electrochemistry measurement. Electrochemistry measurement measures a chemical reaction as an electric signal, for example, current, voltage, and an amount of charges as the name. The weak point of electrochemistry measurement is in the lowness of the singularity. namely, the matter generated by the chemical reaction -- electrochemistry -- even if it is the measurable matter -- the inside of a measurement sample -- electrochemistry -- an error will be produced shortly after another measurable matter exists In order to compensate the weak point of the lowness of the singularity of this electrochemical measurement, an analysis object and the living body functional matter which can react alternatively and specifically, for example, an enzyme etc., are used for a biosensor. A biosensor has the following two portions at least. One is the specified substance and a portion which reacts alternatively and specifically as the optional-feature section. For example, an enzyme and an antibody are equivalent to this. Another is a portion which changes the chemical reaction of the optional-feature section into an electric signal as the signal transformation section. It is mainly equivalent to an electrode. measurement of the analysis object using the biosensor -- direct -- various kinds, such as current, voltage, and the amount of charges, -- an electric amount may become the measuring object The biosensor which makes current the measuring object also in this exists mostly.
[0004] A biosensor system is used combining the device which gives potential or measures the current which is the signal of a biosensor, and the biosensor as a test cell to a biosensor. For example, if it is a biosensor system for carrying out the fixed quantity of the lactic-acid concentration in a blood sample The biosensor which makes a lactic acid and enzymes which react specifically, such as a lactic-acid oxidase (LOD) and a lactate dehydrogenase (LDH), the optional-feature section is made into a test cell. The timer function which clocks the time after the reaction of a blood sample, the aforementioned enzyme, etc. starts, The function to impress the potential defined after the defined time progress to a biosensor, The function which measures current after the time progress defined from the aforementioned potential impression start, The correlation of current and lactic-acid concentration, for example, the correlation which memorizes a calibration curve and by which storage was functioned and carried out [aforementioned], For example, it can build by using it combining the device which has the function which functions and displays the result which judges lactic-acid concentration, and which carried out [

aforementioned] the judgment on a display etc. from the current by which measurement was carried out [aforementioned] with the calibration curve. Such a biosensor system does not remain in the fixed quantity of the lactic-acid concentration in a blood sample, but various things are known.
[0005] The biosensor system which measures the glucose concentration of a blood sample using an amperometry is indicated by the Europe patent application public presentation No. 0230472. In this system, the biosensor equipped with the measuring electrode, the reference electrode, and the counter electrode is used as a test cell. The aforementioned electrode is covered in the reagent layer containing the component of a glucose oxidase, potassium ferricyanide, and others. If a blood sample is contacted in a reagent layer and put in, the glucose in a sample will react with potassium ferricyanide through an operation of a glucose oxidase, and will form a potassium ferrocyanide. If voltage is impressed to an electrode after that, the current proportional to the concentration of the potassium ferrocyanide which reverse reaction produced and was produced at the first reaction will flow. The measured value of this current supposes that it corresponds to the concentration of the glucose in a sample.
[0006] Generally in electrochemistry measurement, the technique of impressing potential in square wave to time, and measuring the current over it is known as a potential step method. It is the meaning of square wave potential impression" impressing the fixed potential which is in a moment substantially, and continuing impressing the account of back to front fixed potential to "time saying here. By such potential step method, the current and the transient-decay current which are decreased with the passage of time after impressing potential are observed. Since it is dependent on time, this transient-decay current can be expressed as a function of time. other ** -- this transient-decay current is dependent also on analysis object concentration Therefore, this transient-decay current can be expressed also as a function of analysis object concentration. The transient-decay current can be expressed as a function of time and analysis object concentration. Therefore, when the character of a sample, sample temperature, electrode area, impression potential, etc. are kept constant as a factor of others which affect the transient-decay current, the transient-decay current is set to I and t and analysis object concentration are set to C for time, it can express I= (t, C). I (t, C) can express the transient-decay current measured to the same timing as a function of only analysis object concentration, if it expresses that I is the function of t and C, Time t can be kept constant and it will put in another way. It will be set to I(t= regularity, C) =I (C) if expressed with a formula. This principle is used in the conventional biosensor system. It decides on the time of an amperometry beforehand and current and the nature concentration of an analysis object are associated using calibration-curve I (C) which shows the current at that time, and the correlation of analysis object concentration. At this time, an amperometry is only 1 time. [0007] The biosensor system which performs the amperometry of multiple times is indicated by the patent official report No. 2651278. In this invention, it is utilizing for detection of the information acquired by performing an amperometry two or more times of an unusual sample supply state. It is going to acquire information other than analysis object concentration from the relation between the current in not the size itself but different time of current. In other words, it is the attempt which is going to acquire information from function [of time] I (t) rather from Current I. In this system, the index of whether the system is working normally has been obtained by performing two continuous amperometries and comparing the ratio of both measurement current with the ratio of the inverse number of the square root of the time when the measurement was performed. This is based on the theoretical formula of I (t, C) in the potential step method under the conditions of having analysis object concentration distribution uniformity and no sample churning being proportional to the inverse number of the square root of time (t) in the state just before applying potential to the amount half infinity of samples, and an electrode. For example, when a blood sample is not wearing the whole front face of a detection electrode and a reaction zone hydrates before a test or during a test, A blood sample by leak arising with furthermore, the index obtained by comparing the ratio of the current acquired by two aforementioned measurement which carries out continuation with the ratio of the inverse number of the square root of the time when the measurement was performed when an error produced not only the electrode in a reaction zone but the exterior of a reaction zone in a wrap case at a reading Suppose that it can distinguish that the error has arisen.

[0008] The method using control liquid as other conventional technology for confirming whether the biosensor system is working normally is learned. Somewhat unlike invention of the patent official report No. 2651278 indication, this technology aims at the check of whether the device [in / a biosensor system / in the purpose] is mainly working normally. The method of the system check using control liquid is explained by making the biosensor system for carrying out the fixed quantity of the glucose concentration of a blood sample to below into an example.
[0009] Control liquid is prepared by known glucose concentration and it is investigated beforehand whether a normal system carries out what response, for example, a glucose concentration display. Furthermore, in order to bring close to the character of a blood sample, there are also what adds a water soluble polymer and is increasing viscosity, and a thing to which coloring matter is added in control liquid. A system-usage person measures the aforementioned control liquid with the same means as the time of measuring a blood sample periodically if needed. And it judges whether a system response is normal. The judgment with a normal system response depends on comparing with an actual system-response value the normal range displayed on a container, an appending document, etc. of control liquid. For example, when a normal range measures the control liquid which is 70 - 120 mg/dL, if system response values are 80 mg/dL, 110 mg/dL, etc., it will judge with the system working normally, and if system response values are 50 mg/dL, 140 mg/dL, etc., it will judge with a system being unusual. Thus, it judges whether the biosensor system is working normally.
[0010]
[Problem(s) to be Solved by the Invention] By the check method of the biosensor system using control liquid, as mentioned above Since it is the same only by there being a display of the nature concentration of an analysis object as a system response even if it is the case where control liquid is measured for a system check, and it is measurement of usual samples, such as a blood sample There was a problem that it could not distinguish what the user used as a sample and whether it is the result of measuring usual samples, such as a blood sample, for whether it is the result of measuring control liquid if not recognized. For this reason, it is incorrect-recognized as it being the result of, for example, measuring control liquid, in spite of having been the result of measuring usual samples, such as a blood sample. or although a system is normal, judge with it being unusual, or Or it has been incorrect-recognized as it being the result of measuring usual samples, such as a blood sample, in spite of having been the result of judging with it being normal or measuring control liquid, although the system was unusual, and there was a possibility of drawing the diagnosis which was wrong in the doctor. It **** and was serious in the danger that the diagnosis mistaken by case like especially the latter based on mistaken blood test data will be drawn.
[0011] Moreover, in case it judged whether a system is normal, the measurement result of a normal range and control liquid displayed on the control liquid container, the appending document, etc. needed to be compared, and work was complicated. When a system check must be performed over ****, or when performing a system check using the control liquid of two or more concentration, if it was system check line trap ******, it was complicated at especially the case where there is nothing to many biosensor systems etc.
[0012] When the purpose of this invention does not hang a burden on a user but measures control liquid, a system discriminates from it being not a usual sample but control liquid, such as a blood sample, automatically, and it displays this, and offers the method for building the biosensor system which can prevent the incorrect recognition by the side of a user. It is offering the method for building the biosensor system which can know the result of a system check with control liquid, without combining and being accompanied by complicated work.
[0013]
[Means for Solving the Problem] this invention is the method of computing the ratio of the current which is the method used for the biosensor system which carries out a fixed quantity, and measured the analysis object concentration of a liquid sample, and its time differential, and performing sample discrimination by making this into an index, by measuring current. The time differential of current which carried out [aforementioned] measurement may be the differential in the strict meaning obtained from the

measurement data which continues in analog, and it may be computed so that it may become a differential value from measurement data discontinuous in digital one substantially. For example, current can be measured at intervals of comparatively short time, and it can consider as time differential with both difference. That is, in the definition formula of time differential, and lim(deltat->0) ={I(t+deltat)-I(t)}/deltat, since deltat must be infinitesimal, although it is impossible, from the current measured by interval deltat of comparatively short time, I hear that asking for strict time differential from discontinuous measurement can ask for time differential in approximation, and there is. Moreover, when deltat cannot be shortened so much in a certain reason, measurement of three or more points can be performed, the moving average of those differences can be taken, and it can ask for time differential in approximation. In this way, the ratio of the obtained time differential and current is taken and it considers as the index of sample discrimination. By request, the ratios of time differential and current may be differential/current, may be current/differential and may include a constant in these in four operations. There is no difference essential to them and how to choose the comparison value described later only differs. Time differential may be approximation-differential (deltaI/deltat) even if it is strict differential (dI/dt). In case the index of sample discrimination is computed, even if the ratio of current and time differential is {I/(dI/dt)} or {I/(deltaI/deltat)} {(dI/dt)/I} or {(deltaI/deltat)/I} is sufficient -- carrying out -- these ratios -- alpha, a, and b -- as a certain constant -- etc. (a **** b) etc. -- ****** -- it is good It is because how to take the comparison value which an essential difference does not have and is described later only differs although chosen as an index of sample discrimination of which [above].

[0014] As a method for obtaining other indexes for sample discrimination, multiple-times calculation of the ratio of the aforementioned current and its time differential can be carried out, and those differences can also be taken. the ratio of current and its time differential -- carrying out n piece (n times) calculation of the alpha, the k-th [the] and the 1st can be set to alphak and alpha1, and these differences can also be expressed as deltaalpha, then deltaalpha(k, l) =alpha k-alpha 1 However, the parenthesis of deltaalpha (k, l) expresses that they are the k-th and the l-th difference of alpha. Even if it does not interfere even if it changes a sign into obtaining the index of sample discrimination also in this case, and it is deltaalpha(k, l) =alpha k-alpha 1, you may be deltaalpha(k, l) =alpha1-alphak. Moreover, a constant may be included in four operations and it is good also considering {c(deltaalpha)**d} as an index for sample discrimination considering c and d as a certain constant. It is because how to take the comparison value which an essential difference does not have and is described later only differs although chosen as an index of sample discrimination of which [above].

[0015] By comparing with a comparison value the index obtained by method which has so far been explained, it can discriminate from a sample. Since the aforementioned comparison value is a value calculated experimentally, in order to discriminate from a sample using this method, a sample must investigate in advance what index value is taken. as an index -- the ratio of current and its time differential -- what is necessary is just to choose alpha 0 as the comparison value as less than Aalpha0 sample and a threshold that Balpha0 or more samples alpha value is observed, in case it discriminates from Sample A and Sample B noting that alpha is used Thus, if the decided comparison value is beforehand stored in a system, sample discrimination will be attained by comparison with the index value and comparison value which are observed at the time of actual measurement. If discrimination is possible, it is easy to display the result on the aforementioned system.

[0016] When the liquid sample measured is based on blood or control liquid, after discriminating from it being control liquid using this method, the judgment result of a system check can also be displayed collectively. A judgment result may be indicated alternative formulas, such as O, x and O.K., and NG, and control High, 270 mg/dl-OK, etc. may display it concretely.

[0017]

[Embodiments of the Invention]

[Example] Hereafter, a concrete example explains this invention in more detail. As an example of a biosensor system, the glucose sensor system which carries out the fixed quantity of the glucose concentration of a blood sample is explained. The glucose sensor which is the test cell of a glucose sensor

system used the thing of the following composition. On the insulating substrate which consists of a PET (polyethylene terephthalate), the electrode system (carbon) and electric insulation layer containing a lead (silver), an operation pole, and a counter electrode are formed of screen-stencil. The electric insulation layer set constant the area for an outcrop of an operation pole and a counter electrode, and has covered the lead partially. Thus, on the formed electrode section, the carboxymethyl-cellulose (CMC) layer which is a hydrophilic macromolecule is formed. Furthermore, on this CMC layer, the enzyme and mediator layer which consist of potassium ferricyanide as the glucose oxidase (GOD) and electron carrier (mediator) as an enzyme are formed. (A CMC layer, an enzyme, and a mediator layer are combined below, and a reaction layer is called.) The insertion which consists of covering and a spacer is formed further, and if a sample touches to an insertion, the sample of about 3microL will be supplied to a reaction layer and an electrode system by capillarity as a constant rate. On the other hand, as measuring equipment, a general-purpose potentiostat and BAS100 B/W (product made from BAS) were used. If it is the usual biosensor system, although the device of it exclusive use will be put together and used for one test cell, the general-purpose device is used in this example. However, even if it is a simple device using known technology, reappearance implementation of this example is easy.

[0018] Control liquid 1, control liquid 2, and blood were used for the measurement sample. Control liquid 1 is a benzoic acid and the solution by which red No. 2 and the glucose were added as coloring matter as antiseptics. Two kinds, 97 mg/dl and 325 mg/dl, were used for glucose concentration. The character of control liquid 1 was close to water, and was comparatively low. [of viscosity] Control liquid 2 is solution of a polyvinyl pyrrolidone (PVP) and a glucose which is a water soluble polymer. Two kinds, 83 mg/dl and 256 mg/dl, were used for glucose concentration. The character of control liquid 2 had comparatively high viscosity. Six kinds from which corpuscle constituent concentration and glucose concentration differ were used for blood. Corpuscle constituent concentration (hematocrit shows.) and glucose concentration were (30%, 102 mg/dl), (44%, 101 mg/dl), (58%, 103 mg/dl), (28%, 268 mg/dl), (45%, 263 mg/dl), and (62%, 266 mg/dl). Moreover, the heparin sodium was added as an anticoagulant. These samples were supplied to the reaction layer and electrode system of a test cell, and it put, without impressing potential for 25 seconds. In the meantime, the glucose in a sample reacts with the potassium ferricyanide of a reaction layer through an operation of a glucose oxidase, and generates a potassium ferrocyanide. And for 5 seconds after after 25 seconds, between the operation pole and the counter electrode, the fixed potential of 500mV was impressed in square wave to time, and current was measured at intervals of 0.1 seconds. The current observed at this time is based on the reverse reaction of potassium ferricyanide from a potassium ferrocyanide. Since this current is proportional to the concentration of the potassium ferrocyanide produced at the first reaction, the measured value of this current is equivalent to the glucose concentration of a sample. Moreover, this current is the transient-decay current decreased with the time progress from potential impression. The relation between time and impression potential is shown in drawing 1 . Moreover, the transient-decay current at the time of measuring what is glucose concentration 97 mg/dl of control liquid 1 to drawing 2 is illustrated. The time basis of a horizontal axis is a second and makes 0 second the moment that potential is impressed.

[0019] (Operation 1) The operation value for sample discrimination is acquired by the method shown below. In addition, although the time from voltage impression uses the current of 4 seconds after in this example, the current of the time which was not limited to this and was suitable for sample discrimination can be used. First, the difference which reduced the current 4.1 seconds after the value 4 seconds after the measured transient-decay current was taken, and it considered as the time differential of after [4 seconds] current. This is replaced with infinitesimal deltat in the definition formula of time differential, and lim(deltat->0) ={I(t+deltat)-I(t)}/deltat, takes deltat= 0.1 seconds, and is equivalent to what replaced the sign. In this way, the value of the ratio which used a denominator and after [4 seconds] current as the molecule for the obtained time differential was taken, and this was made into the discrimination index alpha 4. It is shown that 4 of the suffix of alpha 4 is the index obtained from after [4 seconds] current. The value of the index alpha 4 in each sample is shown in Table 1. In addition, the data of control liquid 1 and control liquid 2 are as a result of a measurement size 30, respectively, and the data of blood are as a

result of a measurement size 90. alpha4 value according to sample is shown in Table 1.
[Table 1]

	平均値	最小値	最大値
コントロール液1	122	114	134
コントロール液2	75	65	85
血液	83	54	104

It can discriminate from both by using a small thing as blood, being able to use as control liquid 1 what shows alpha4 larger value than the comparison value 110 if 110 is chosen from Table 1 as a comparison value since the minimum value of control liquid 1 is larger than the maximum of blood for example. Similarly, it can discriminate also from control liquid 1 and control liquid 2. However, in control liquid 2 and blood, since the maximum of control liquid 2 is larger than the minimum value of blood, discrimination is impossible.
[0020] (Operation 2) the ratio of the current acquired next like an operation 1, and its time differential -- it asks for two or more alphat (mind of alpha obtained from after [t seconds] current), and the case where those differences are made into the index value for sample discrimination is explained alpha0.5 and alpha 2 are calculated about 0.5 seconds and 2 seconds. If expressed with a formula, it will be alpha0.5=I0.5/(I0.5-I0.6), respectively.
It can express alpha2=I2/(I2-I2.1). It asks for these, next these differences are taken. In this example, the difference which reduced alpha 2 to alpha 0.5 was taken. If the operation in this example is expressed with a formula expressing the difference of alphat1 for alphat2 for t 2 seconds, and t 1 second like deltaalpha (t1, t2), it will become deltaalpha(0. 5 2) =alpha2-alpha0.5={I2/(I2-I2.1)}-{I0.5/(I0.5-I0.6)}. The value of deltaalpha (0. 5 2) in each sample is shown in Table 2. In addition, the data made into the basis are the same as that of what was used for the operation 1, the data of control liquid 2 are as a result of a measurement size 30, and the data of blood are as a result of a measurement size 90.
[Table 2]

	平均値	最小値	最大値
コントロール液1	42	39	44
コントロール液2	25	21	29
血液	35	33	39

It can discriminate from both by using a large thing as blood, being able to use as control liquid 2 what shows deltaalpha (0. 5 2) value smaller than the comparison value 30 if 30 is chosen from Table 2 as a comparison value since the maximum of control liquid 2 is smaller than the minimum value of blood for example.
[0021] It becomes possible from the above result to discriminate from three samples of control liquid 1, control liquid 2, and blood. That is, with [the value of deltaalpha (0. 5 2)] 30 [less than], in this example, it judges with it being control liquid 2, with 30 [or more], it judges with their being blood or control 1, and with [further with / the value of alpha 4 / 110 / less than / in blood] 110 [or more], it can judge with it being control liquid 1. By this, it is able to discriminate from three samples of control liquid 1, control liquid 2, and blood.
[0022] Since sample discrimination is attained by method which has so far been described, it is easy to build the biosensor system which can display a discrimination result. Moreover, as a result of the system check using control liquid, since it can discriminate from a display that the sample measured by this method is control liquid, if the normal-range current value of control liquid is beforehand stored in a system, it will also become possible to display the result of the amperometry for investigating concentration, and the result which compared the normal-range current value.
[0023]
[Effect of the Invention] By using the method of this invention, when a burden is not hung on a user but control liquid is measured, it can discriminate from devices being not a usual sample but control liquid, such as a blood sample, automatically, and this can be displayed, and the biosensor system which can

prevent the incorrect recognition by the side of a user can be built. It combines, and the biosensor system which can know the result of a system check with control liquid can be built, without being accompanied by complicated work.
[0024]

[Translation done.]

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TECHNICAL FIELD

[The technical field to which invention belongs] this invention relates to the method for measuring the analysis object concentration contained in a liquid sample. It is related with the method for [which carries out the fixed quantity of the concentration, such as a glucose and cholesterol for example by the amperometry] being contained especially in body fluid, such as blood.

[Translation done.]

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PRIOR ART

[Description of the Prior Art] Conventionally, the biosensor is known as what carries out the fixed quantity of the analysis object concentration in living body samples, such as a liquid sample, especially blood, simple and quickly.

[0003] A biosensor is one sort of the device for performing electrochemistry measurement. Electrochemistry measurement measures a chemical reaction as an electric signal, for example, current, voltage, and an amount of charges as the name. The weak point of electrochemistry measurement is in the lowness of the singularity. namely, the matter generated by the chemical reaction -- electrochemistry -- even if it is the measurable matter -- the inside of a measurement sample -- electrochemistry -- an error will be produced shortly after another measurable matter exists In order to compensate the weak point of the lowness of the singularity of this electrochemical measurement, an analysis object and the living body functional matter which can react alternatively and specifically, for example, an enzyme etc., are used for a biosensor. A biosensor has the following two portions at least. One is the specified substance and a portion which reacts alternatively and specifically as the optional-feature section. For example, an enzyme and an antibody are equivalent to this. Another is a portion which changes the chemical reaction of the optional-feature section into an electric signal as the signal transformation section. It is mainly equivalent to an electrode. measurement of the analysis object using the biosensor -- direct -- various kinds, such as current, voltage, and the amount of charges, -- an electric amount may become the measuring object The biosensor which makes current the measuring object also in this exists mostly.

[0004] A biosensor system is used combining the device which gives potential or measures the current which is the signal of a biosensor, and the biosensor as a test cell to a biosensor. For example, the lactic-acid oxidase which will react specifically with a lactic acid if it is a biosensor system for carrying out the fixed quantity of the lactic-acid concentration in a blood sample (LOD), The biosensor which makes enzymes, such as a lactate dehydrogenase (LDH), the optional-feature section is made into a test cell. The timer function which clocks the time after the reaction of a blood sample, the aforementioned enzyme, etc. starts, The function to impress the potential defined after the defined time progress to a biosensor, The function which measures current after the time progress defined from the aforementioned potential impression start, The correlation of current and lactic-acid concentration, for example, the correlation which memorizes a calibration curve and by which storage was functioned and carried out [aforementioned], For example, it can build by using it combining the device which has the function which functions and displays the result which judges lactic-acid concentration, and which carried out [aforementioned] the judgment on a display etc. from the current by which measurement was carried out [aforementioned] with the calibration curve. Such a biosensor system does not remain in the fixed quantity of the lactic-acid concentration in a blood sample, but various things are known.

[0005] The biosensor system which measures the glucose concentration of a blood sample using an amperometry is indicated by the Europe patent application public presentation No. 0230472. In this system, the biosensor equipped with the measuring electrode, the reference electrode, and the counter electrode is used as a test cell. The aforementioned electrode is covered in the reagent layer containing

the component of a glucose oxidase, potassium ferricyanide, and others. If a blood sample is contacted in a reagent layer and put in, the glucose in a sample will react with potassium ferricyanide through an operation of a glucose oxidase, and will form a potassium ferrocyanide. If voltage is impressed to an electrode after that, the current proportional to the concentration of the potassium ferrocyanide which reverse reaction produced and was produced at the first reaction will flow. The measured value of this current supposes that it corresponds to the concentration of the glucose in a sample.

[0006] Generally in electrochemistry measurement, the technique of impressing potential in square wave to time, and measuring the current over it is known as a potential step method. It is the meaning of square wave potential impression" impressing the fixed potential which is in a moment substantially, and continuing impressing the account of back to front fixed potential to "time saying here. By such potential step method, the current and the transient-decay current which are decreased with the passage of time after impressing potential are observed. Since it is dependent on time, this transient-decay current can be expressed as a function of time. other ** -- this transient-decay current is dependent also on analysis object concentration Therefore, this transient-decay current can be expressed also as a function of analysis object concentration. The transient-decay current can be expressed as a function of time and analysis object concentration. Therefore, when the character of a sample, sample temperature, electrode area, impression potential, etc. are kept constant as a factor of others which affect the transient-decay current, the transient-decay current is set to I and t and analysis object concentration are set to C for time, it can express I= (t, C). I (t, C) can express the transient-decay current measured to the same timing as a function of only analysis object concentration, if it expresses that I is the function of t and C, Time t can be kept constant and it will put in another way. It will be set to I(t= regularity, C) =I (C) if expressed with a formula. This principle is used in the conventional biosensor system. It decides on the time of an amperometry beforehand and current and the nature concentration of an analysis object are associated using calibration-curve I (C) which shows the current at that time, and the correlation of analysis object concentration. At this time, an amperometry is only 1 time.

[0007] The biosensor system which performs the amperometry of multiple times is indicated by the patent official report No. 2651278. In this invention, it is utilizing for detection of the information acquired by performing an amperometry two or more times of an unusual sample supply state. It is going to acquire information other than analysis object concentration from the relation between the current in not the size itself but different time of current. In other words, it is the attempt which is going to acquire information from function [of time] I (t) rather from Current I. In this system, the index of whether the system is working normally has been obtained by performing two continuous amperometries and comparing the ratio of both measurement current with the ratio of the inverse number of the square root of the time when the measurement was performed. This is based on the theoretical formula of I (t, C) in the potential step method under the conditions of having analysis object concentration distribution uniformity and no sample churning being proportional to the inverse number of the square root of time (t) in the state just before applying potential to the amount half infinity of samples, and an electrode. For example, when a blood sample is not wearing the whole front face of a detection electrode and a reaction zone hydrates before a test or during a test, Furthermore, the index by which the blood sample was obtained by leak arising by comparing the ratio of the current acquired by two aforementioned measurement which carries out continuation when an error produced not only the electrode in a reaction zone but the exterior of a reaction zone in a wrap case at a reading with the ratio of the inverse number of the square root of the time when the measurement was performed. Suppose that it can distinguish that the error has arisen.

[0008] The method using control liquid as other conventional technology for confirming whether the biosensor system is working normally is learned. Somewhat unlike invention of the patent official report No. 2651278 indication, this technology aims at the check of whether the device [in / a biosensor system / in the purpose] is mainly working normally. The method of the system check using control liquid is explained by making the biosensor system for carrying out the fixed quantity of the glucose concentration of a blood sample to below into an example.

[0009] Control liquid is prepared by known glucose concentration and it is investigated beforehand

whether a normal system carries out what response, for example, a glucose concentration display. Furthermore, in order to bring close to the character of a blood sample, there are also what adds a water soluble polymer and is increasing viscosity, and a thing to which coloring matter is added in control liquid. A system-usage person measures the aforementioned control liquid with the same means as the time of measuring a blood sample periodically if needed. And it judges whether a system response is normal. The judgment with a normal system response depends on comparing with an actual system-response value the normal range displayed on a container, an appending document, etc. of control liquid. For example, when a normal range measures the control liquid which is 70 - 120 mg/dL, if system response values are 80 mg/dL, 110 mg/dL, etc., it will judge with the system working normally, and if system response values are 50 mg/dL, 140 mg/dL, etc., it will judge with a system being unusual. Thus, it judges whether the biosensor system is working normally.

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EFFECT OF THE INVENTION

[Effect of the Invention] By using the method of this invention, when a burden is not hung on a user but control liquid is measured, it can discriminate from devices being not a usual sample but control liquid, such as a blood sample, automatically, and this can be displayed, and the biosensor system which can prevent the incorrect recognition by the side of a user can be built. It combines, and the biosensor system which can know the result of a system check with control liquid can be built, without being accompanied by complicated work.

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TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] By the check method of the biosensor system using control liquid, as mentioned above Since it is the same only by there being a display of the nature concentration of an analysis object as a system response even if it is the case where control liquid is measured for a system check, and it is measurement of usual samples, such as a blood sample There was a problem that it could not distinguish what the user used as a sample and whether it is the result of measuring usual samples, such as a blood sample, for whether it is the result of measuring control liquid if not recognized. For this reason, it is incorrect-recognized as it being the result of, for example, measuring control liquid, in spite of having been the result of measuring usual samples, such as a blood sample. or although a system is normal, judge with it being unusual, or Or it has been incorrect-recognized as it being the result of measuring usual samples, such as a blood sample, in spite of having been the result of judging with it being normal or measuring control liquid, although the system was unusual, and there was a possibility of drawing the diagnosis which was wrong in the doctor. It **** and was serious in the danger that the diagnosis mistaken by case like especially the latter based on mistaken blood test data will be drawn.
[0011] Moreover, in case it judged whether a system is normal, the measurement result of a normal range and control liquid displayed on the control liquid container, the appending document, etc. needed to be compared, and work was complicated. When a system check must be performed over ****, or when performing a system check using the control liquid of two or more concentration, if it was system check line trap ******, it was complicated at especially the case where there is nothing to many biosensor systems etc.
[0012] When the purpose of this invention does not hang a burden on a user but measures control liquid, a system discriminates from it being not a usual sample but control liquid, such as a blood sample, automatically, and it displays this, and offers the method for building the biosensor system which can prevent the incorrect recognition by the side of a user. It is offering the method for building the biosensor system which can know the result of a system check with control liquid, without combining and being accompanied by complicated work.

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3.In the drawings, any words are not translated.

MEANS

[Means for Solving the Problem] this invention is the method of computing the ratio of the current which is the method used for the biosensor system which carries out a fixed quantity, and measured the analysis object concentration of a liquid sample, and its time differential, and performing sample discrimination by making this into an index, by measuring current. The time differential of current which carried out [aforementioned] measurement may be the differential in the strict meaning obtained from the measurement data which continues in analog, and it may be computed so that it may become a differential value from measurement data discontinuous in digital one substantially. For example, current can be measured at intervals of comparatively short time, and it can consider as time differential with both difference. That is, in the definition formula of time differential, and lim(deltat->0) ={I(t+deltat)-I(t)}/deltat, since deltat must be infinitesimal, although it is impossible, from the current measured by interval deltat of comparatively short time, I hear that asking for strict time differential from discontinuous measurement can ask for time differential in approximation, and there is. Moreover, when deltat cannot be shortened so much in a certain reason, measurement of three or more points can be performed, the moving average of those differences can be taken, and it can ask for time differential in approximation. In this way, the ratio of the obtained time differential and current is taken and it considers as the index of sample discrimination. By request, the ratios of time differential and current may be differential/current, may be current/differential and may include a constant in these in four operations. There is no difference essential to them and how to choose the comparison value described later only differs. Time differential may be approximation-differential (deltaI/deltat) even if it is strict differential (dI/dt). In case the index of sample discrimination is computed, even if the ratio of current and time differential is {I/(dI/dt)} or {I/(deltaI/deltat)} {(dI/dt)/I} or {(deltaI/deltat)/I} is sufficient -- carrying out -- these ratios -- alpha, a, and b -- as a certain constant -- etc. (a **** b) etc. -- ****** -- it is good It is because how to take the comparison value which an essential difference does not have and is described later only differs although chosen as an index of sample discrimination of which [above].
[0014] As a method for obtaining other indexes for sample discrimination, multiple-times calculation of the ratio of the aforementioned current and its time differential can be carried out, and those differences can also be taken. the ratio of current and its time differential -- carrying out n piece (n times) calculation of the alpha, the k-th [the] and the 1st can be set to alphak and alpha1, and these differences can also be expressed as deltaalpha, then deltaalpha(k, l) =alpha k-alpha 1 However, the parenthesis of deltaalpha (k, l) expresses that they are the k-th and the l-th difference of alpha. Even if it does not interfere even if it changes a sign into obtaining the index of sample discrimination also in this case, and it is deltaalpha(k, l) =alpha k-alpha 1, you may be deltaalpha(k, l) =alpha1-alphak. Moreover, a constant may be included in four operations and it is good also considering {c(deltaalpha)**d} as an index for sample discrimination considering c and d as a certain constant. It is because how to take the comparison value which an essential difference does not have and is described later only differs although chosen as an index of sample discrimination of which [above].
[0015] By comparing with a comparison value the index obtained by method which has so far been

explained, it can discriminate from a sample. Since the aforementioned comparison value is a value calculated experimentally, in order to discriminate from a sample using this method, a sample must investigate in advance what index value is taken. as an index -- the ratio of current and its time differential -- what is necessary is just to choose alpha 0 as the comparison value as less than Aalpha0 sample and a threshold that Balpha0 or more samples alpha value is observed, in case it discriminates from Sample A and Sample B noting that alpha is used Thus, if the decided comparison value is beforehand stored in a system, sample discrimination will be attained by comparison with the index value and comparison value which are observed at the time of actual measurement. If discrimination is possible, it is easy to display the result on the aforementioned system.

[0016] When the liquid sample measured is based on blood or control liquid, after discriminating from it being control liquid using this method, the judgment result of a system check can also be displayed collectively. A judgment result may be indicated alternative formulas, such as O, x and O.K., and NG, and control High, 270 mg/dl-OK, etc. may display it concretely.

[0017]

[Embodiments of the Invention]

[Translation done.]

* NOTICES *

EXAMPLE

[Example] Hereafter, a concrete example explains this invention in more detail. As an example of a biosensor system, the glucose sensor system which carries out the fixed quantity of the glucose concentration of a blood sample is explained. The glucose sensor which is the test cell of a glucose sensor system used the thing of the following composition. On the insulating substrate which consists of a PET (polyethylene terephthalate), the electrode system (carbon) and electric insulation layer containing a lead (silver), an operation pole, and a counter electrode are formed of screen-stencil. The electric insulation layer set constant the area for an outcrop of an operation pole and a counter electrode, and has covered the lead partially. Thus, on the formed electrode section, the carboxymethyl-cellulose (CMC) layer which is a hydrophilic macromolecule is formed. Furthermore, on this CMC layer, the enzyme and mediator layer which consist of potassium ferricyanide as the glucose oxidase (GOD) and electron carrier (mediator) as an enzyme are formed. (A CMC layer, an enzyme, and a mediator layer are combined below, and a reaction layer is called.) The insertion which consists of covering and a spacer is formed further, and if a sample touches to an insertion, the sample of about 3microL will be supplied to a reaction layer and an electrode system by capillarity as a constant rate. On the other hand, as measuring equipment, a general-purpose potentiostat and BAS100 B/W (product made from BAS) were used. If it is the usual biosensor system, although the device of it exclusive use will be put together and used for one test cell, the general-purpose device is used in this example. However, even if it is a simple device using known technology, reappearance implementation of this example is easy.
[0018] Control liquid 1, control liquid 2, and blood were used for the measurement sample. Control liquid 1 is a benzoic acid and the solution by which red No. 2 and the glucose were added as coloring matter as antiseptics. Two kinds, 97 mg/dl and 325 mg/dl, were used for glucose concentration. The character of control liquid 1 was close to water, and was comparatively low. [of viscosity] Control liquid 2 is solution of a polyvinyl pyrrolidone (PVP) and a glucose which is a water soluble polymer. Two kinds, 83 mg/dl and 256 mg/dl, were used for glucose concentration. The character of control liquid 2 had comparatively high viscosity. Six kinds from which corpuscle constituent concentration and glucose concentration differ were used for blood. Corpuscle constituent concentration (hematocrit shows.) and glucose concentration were (30%, 102 mg/dl), (44%, 101 mg/dl), (58%, 103 mg/dl), (28%, 268 mg/dl), (45%, 263 mg/dl), and (62%, 266 mg/dl). Moreover, the heparin sodium was added as an anticoagulant. These samples were supplied to the reaction layer and electrode system of a test cell, and it put, without impressing potential for 25 seconds. In the meantime, the glucose in a sample reacts with the potassium ferricyanide of a reaction layer through an operation of a glucose oxidase, and generates a potassium ferrocyanide. And for 5 seconds after after 25 seconds, between the operation pole and the counter electrode, the fixed potential of 500mV was impressed in square wave to time, and current was measured at intervals of 0.1 seconds. The current observed at this time is based on the reverse reaction of potassium ferricyanide from a potassium ferrocyanide. Since this current is proportional to the concentration of the potassium ferrocyanide produced at the first reaction, the measured value of this current is equivalent to the glucose concentration of a sample. Moreover, this current is the

transient-decay current decreased with the time progress from potential impression. The relation between time and impression potential is shown in drawing 1 . Moreover, the transient-decay current at the time of measuring what is glucose concentration 97 mg/dl of control liquid 1 to drawing 2 is illustrated. The time basis of a horizontal axis is a second and makes 0 second the moment that potential is impressed.

[0019] (Operation 1) The operation value for sample discrimination is acquired by the method shown below. In addition, although the time from voltage impression uses the current of 4 seconds after in this example, the current of the time which was not limited to this and was suitable for sample discrimination can be used. First, the difference which reduced the current 4.1 seconds after the value 4 seconds after the measured transient-decay current was taken, and it considered as the time differential of after [4 seconds] current. This is replaced with infinitesimal deltat in the definition formula of time differential, and lim(deltat->0) ={I(t+deltat)-I(t)}/deltat, takes deltat= 0.1 seconds, and is equivalent to what replaced the sign. In this way, the value of the ratio which used a denominator and after [4 seconds] current as the molecule for the obtained time differential was taken, and this was made into the discrimination index alpha 4. It is shown that 4 of the suffix of alpha 4 is the index obtained from after [4 seconds] current. The value of the index alpha 4 in each sample is shown in Table 1. In addition, the data of control liquid 1 and control liquid 2 are as a result of a measurement size 30, respectively, and the data of blood are as a result of a measurement size 90. alpha4 value according to sample is shown in Table 1.

[Table 1]

	平均値	最小値	最大値
コントロール液1	122	114	134
コントロール液2	75	65	85
血液	83	54	104

It can discriminate from both by using a small thing as blood, being able to use as control liquid 1 what shows alpha4 larger value than the comparison value 110 if 110 is chosen from Table 1 as a comparison value since the minimum value of control liquid 1 is larger than the maximum of blood for example. Similarly, it can discriminate also from control liquid 1 and control liquid 2. However, in control liquid 2 and blood, since the maximum of control liquid 2 is larger than the minimum value of blood, discrimination is impossible.

[0020] (Operation 2) the ratio of the current acquired next like an operation 1, and its time differential -- it asks for two or more alphat (mind of alpha obtained from after [t seconds] current), and the case where those differences are made into the index value for sample discrimination is explained alpha0.5 and alpha 2 are calculated about 0.5 seconds and 2 seconds. If expressed with a formula, it will be alpha0.5=I0.5/(I0.5-I0.6), respectively.

It can express alpha2=I2/(I2-I2.1). It asks for these, next these differences are taken. In this example, the difference which reduced alpha 2 to alpha 0.5 was taken. If the operation in this example is expressed with a formula expressing the difference of alphat1 for alphat2 for t 2 seconds, and t 1 second like deltaalpha (t1, t2), it will become deltaalpha(0. 5 2) =alpha2-alpha0.5={I2/(I2-I2.1)}-{I0.5/(I0.5-I0.6)}. The value of deltaalpha (0. 5 2) in each sample is shown in Table 2. In addition, the data made into the basis are the same as that of what was used for the operation 1, the data of control liquid 2 are as a result of a measurement size 30, and the data of blood are as a result of a measurement size 90.

[Table 2]

	平均値	最小値	最大値
コントロール液1	42	39	44
コントロール液2	25	21	29
血液	35	33	39

It can discriminate from both by using a large thing as blood, being able to use as control liquid 2 what shows deltaalpha (0. 5 2) value smaller than the comparison value 30 if 30 is chosen from Table 2 as a comparison value since the maximum of control liquid 2 is smaller than the minimum value of blood for example.

[0021] It becomes possible from the above result to discriminate from three samples of control liquid 1, control liquid 2, and blood. That is, with [the value of deltaalpha (0. 5 2)] 30 [less than], in this example, it judges with it being control liquid 2, with 30 [or more], it judges with their being blood or control 1, and with [further with / the value of alpha 4 / 110 / less than / in blood] 110 [or more], it can judge with it being control liquid 1. By this, it is able to discriminate from three samples of control liquid 1, control liquid 2, and blood.

[0022] Since sample discrimination is attained by method which has so far been described, it is easy to build the biosensor system which can display a discrimination result. Moreover, as a result of the system check using control liquid, since it can discriminate from a display that the sample measured by this method is control liquid, if the normal-range current value of control liquid is beforehand stored in a system, it will also become possible to display the result of the amperometry for investigating concentration, and the result which compared the normal-range current value.

[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DESCRIPTION OF DRAWINGS

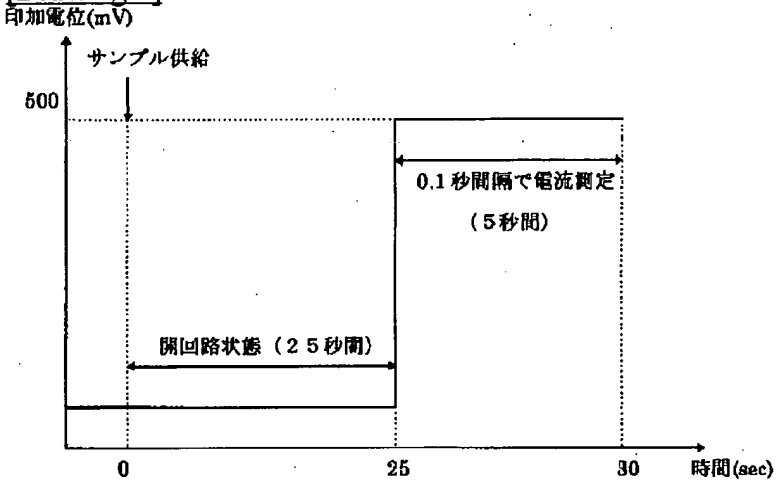
[Brief Description of the Drawings]
[Drawing 1] The relation between time and impression potential is expressed.
[Drawing 2] The transient-decay current at the time of measuring what is glucose concentration 97 mg/dl is shown.

[Translation done.]

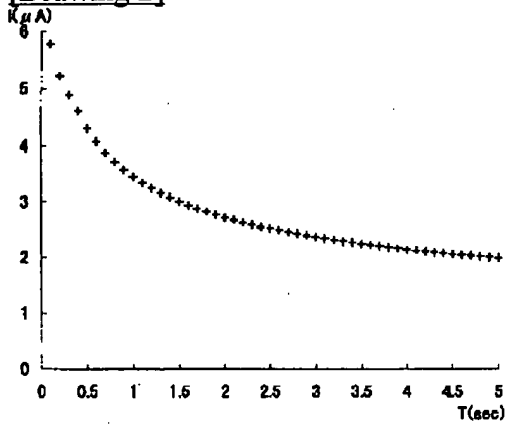
* NOTICES *

DRAWINGS

[Drawing 1]



[Drawing 2]



[Translation done.]